Cerebral blood volume fraction quantification in mice

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Introduction: Noninvasive quantification of regional cerebral blood volume fraction (BVf) by magnetic resonance (MR) imaging has proven successful in mapping brain dysfunction and in testing drug efficacy. Most approaches require intravenous (i.v.) injections of contrast agents [1,2], which presents limitations for mapping BVf longitudinally over time in particular in small animal models such as mice [3]. In this study, we demonstrate that the Rapid Steady State T_1 ($RSST_1$) MR technique, previously used with i.v. injections [1] in rats, can be used with intraperitoneal (i.p.) injections of Gd-DOTA to safely and reliably acquire cerebral BVf maps in mice. To achieve this aim, we compared the signal amplitude after i.v. and i.p. injections of Gd-DOTA in the same mouse.

Material and methods: The mice were imaged in a 47/40 Bruker Biospec USR AV III scanner using a homogenous coil for transmission and a mouse head surface coil for reception. A 3D inversion recovery prepared MDEFT sequence (ParaVision 5.0, one segment, TE = 1.2 ms, TR_{echo} = 6.5 ms, excitation flip angle = 10°, matrix 32 × 32, FOV 15 × 15 mm^2, 8 coronal slices × 0.7 mm) was used. Suitable i.v. and i.p. doses of Gd-DOTA were established in pilot dose experiments carried out on C57BL6J mice by injecting cumulative doses in the range of 0.3 to 1.4 mmol/kg i.v. and 2.5 to 10 mmol/kg i.p. during dynamic $RSST_1$ imaging (TR = 750 ms, T_{inv} = 303 ms) until the additional dose did not change the signal amplitude. To further confirm the $RSST_1$ conditions [1], quantitative T_1 and T_2 maps of mouse brain tissue at 4.7T were acquired and T_1 and T_2 spectroscopic measurements were performed on plasma sampled 30 minutes after i.p. administration of 6 mmol/kg Gd-DOTA to C57BL6 mice (n = 4). These plasma samples were also used in an *in vitro* BVf experiment with the $RSST_1$ technique as described in [1].
NMRI mice (n = 6) were equipped with both a tail vein catheter (26G) and an i.p. catheter (24G) connected to extension lines preloaded with 0.7 mmol/kg and 6 mmol/kg Gd-DOTA, respectively. Prior to Gd-DOTA injection, a proton density weighted image was acquired (TR = 10 s, T_{inv} = 9 s, duration 1 min 20 s) providing the equilibrium signal from the vascular and extravascular compartment for BVf normalization. A dynamic $RSST_1$ scan with a time resolution of 6 s per repetition was acquired over one hour (n = 3) and over 2 hours (n = 3), with the i.v. injection administered at 5 minutes and the i.p. injection administered at 15 minutes into the scan. Once the signal steady state interval after i.p, injection was established, the mice underwent a second experiment for the acquisition of 128 × 128 matrix BVf maps using the MDEFT sequence with 4 segments (TE = 1.7 ms and TR_{echo} = 8.0 ms, total duration ca. 8 minutes) during the signal steady state interval following i.p. administration of Gd-DOTA.
The BVf maps were obtained according to $S_{norm}(t) = (S_{post}(t) - \langle S_{pre} \rangle)/S_0$, where $S_{post}(t)$ is the post contrast signal and $\langle S_{pre} \rangle$ is the average pre contrast signal, while S_0 is the signal in the proton density weighted image. The normalized signal $S_{norm}(t)$ equals the BVf when the contrast agent is confined to the intravascular space and when blood $T_1 < T_{inv}/5 \approx 60$ ms, since the signal is reflecting the thermodynamic equilibrium magnetization of the intravascular compartment [1].

Results and discussion: With a T_1 in the range of 1300 to 1500 ms at 4.7T prior to Gd-DOTA injection, the brain tissue signal is optimally suppressed with TR/T_{inv} = 750/303 ms [1] and reaches thermal equilibrium at T_{inv} = 9 s. An i.p. dose exceeding 6 mmol/kg Gd-DOTA does not further increase the blood signal in $RSST_1$ acquisitions (Fig. 1). Occasional deaths occurred 1 - 2 days after a 10 mmol/kg dose [3], but not after 6 mmol/kg Gd-DOTA. The plasma T_1 and T_2 30 minutes after i.p. administration of 6 mmol/kg were 4.6 ± 0.3 ms and 5.7 ± 0.5 ms, respectively, demonstrating that the intravascular protons relax to thermal equilibrium with little transverse relaxation effects. The *in vitro* BVf experiment yielded an average BVf of 0.0101 ± 0.0001.
The green plot in Fig. 2a shows a typical normalized brain signal $S_{norm}(t)$ from the dynamic $RSST_1$ acquisition and demonstrates that the peak signal after i.v. administration and the steady state signal 15 to 35 minutes after i.p. administration of Gd-DOTA lead to equivalent cerebral BVf measures which are in the order of 0.023 ± 0.003 (n = 6). In Fig. 2b, the $S_{norm}(t)$ from a single pixel located in a large vessel is shown in bright red and yields a BVf of 1. However, regions of interest including ventricles (blue plot in Fig. 2a) exhibit a contrast agent leakage profile, such as typically observed in skin (brown plot in Fig. 2b) or muscle tissue (dark red in Fig. 2b). Approximately 60 minutes after i.p. Gd-DOTA injection the signal increases continuously, in particular in cerebral regions close to ventricles (cf. Fig. 3). Gd-DOTA accumulation in cerebrospinal fluid due to leakage in the choroid plexus is the assumed cause. Fig. 4 shows a typical coronal high resolution (pixel size =117 μm^2) BVf map at bregma -1.8 mm position.

Conclusion: BVf mapping using the $RSST_1$ technique was performed with i.v. and i.p. injections of Gd-DOTA in the same animals. The brain tissue signal amplitude after i.v. injection is equal to the steady state signal amplitude after i.p. injection demonstrating that the signal corresponds to the thermal equilibrium magnetization of the vascular space. In mice, a steady state signal is obtained for a time interval of at least 20 minutes starting 15 minutes after i.p. administration of 6 mmol/kg Gd-DOTA. With the $RSST_1$ MR technique, this time interval can be used for acquiring BVf maps with increased spatial resolution, for determining functional changes of the BVf during the time interval and for testing the vasoactivity of drugs in a pharmacological MR experiment allowing each animal to serve as its own control, before and after drug administration. Compared to i.v. injections, i.p. administration of Gd-DOTA in mice is less traumatic with practically no risk for emboli or hypervolemia, and can therefore be used repeatedly in longitudinal studies such as for monitoring tumor angiogenesis.

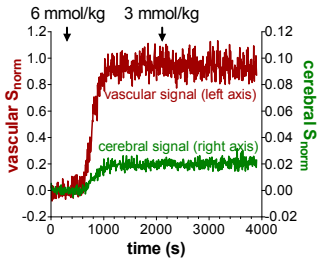
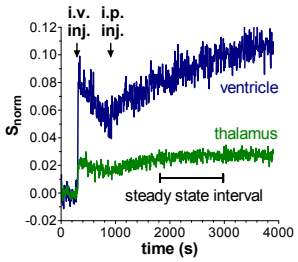


Fig. 1
representative dose experiment



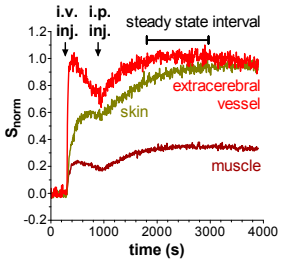


Fig. 2a **b**
typical S_{norm} time course for different regions of interest (n = 1)

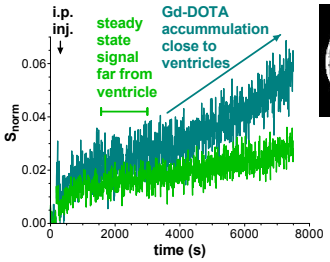


Fig. 3
Gd-DOTA accumulation over time in brain regions close to ventricles

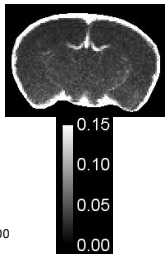


Fig. 4
coronal BVf map

[1] Perles-Barbacaru and Lahrech, J Cereb Blood Flow Metab 2007; [2] Schwarzbauer et al, Magn Reson Med 1993; [3] Moreno et al, NMR Biomed 2006